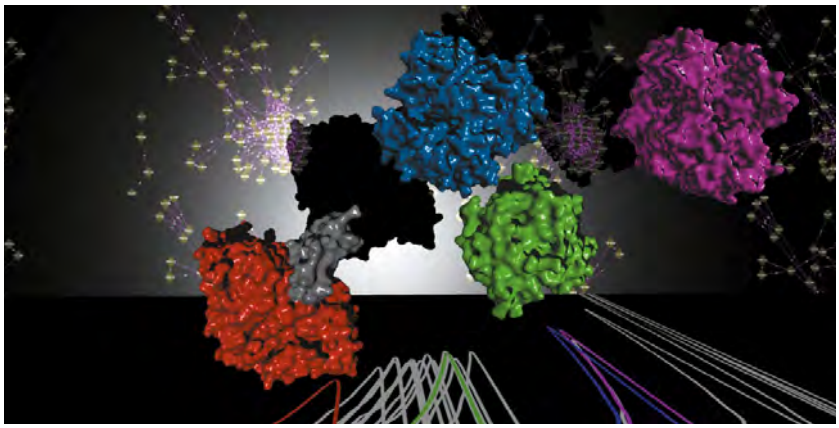


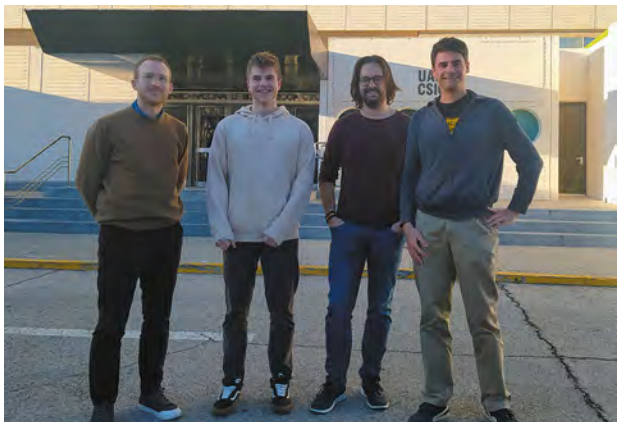
Systems Biology

The successful application of Molecular Biology over the last decades is providing many of the molecular agents underpinning fundamental cellular processes. While this reductionist approach is surely going to be valid in the near future, Biology is now facing a new age, in which questions about how molecular elements act together are of great demand. This is particularly significant for the development of a new Biotechnology based on the rational control of biological processes.

The Systems Biology Department of the Spanish National Biotechnology Center (CNB) tries to promote this new discipline and its Biotechnological applications. Our vision is to bring together researchers with quantitative, computational and experimental backgrounds to understand and engineer complete biological systems. The program is also planned to act as a core unit of a broader Systems Biology initiative at the Spanish National Research Council (CSIC).

HEAD

Florencio Pazos



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Clocks and rulers in life

During the period 2021-2021, different projects have been developed simultaneously. Following our collaboration with the group of Wilfried Meijer at CBMSO (see Meijer *et al*, 2021), we have been working on mathematical models of bacterial conjugation in Gram-positive bacteria. We have also been working on the pattern formation of nitrogen-fixing cells in filamentous cyanobacteria (Figure 1, Casanova-Ferrer 2022), and on models for the regulation by nitrogen of tillering in Green Revolution rice varieties.

Modelling plant growth has been a big topic (Figure 2): in collaboration with the group of Salomé Prat we described the effects of light and temperature on the growth of *Arabidopsis thaliana* (Nieto *et al*, 2022). Furthermore, we have been working towards generalising this model to incorporate circadian rhythms.

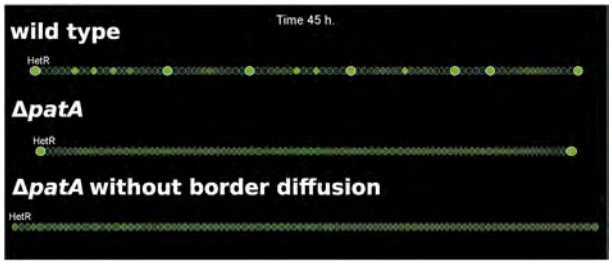
We are also interested in understanding the physics of how cells and plant organs grow. Currently, we are working in collaboration with Pilar Cubas' group on an experimental and modelling project to understand the control of dormancy in *Arabidopsis*' axillary buds.

In this period, James Pelletier, a postdoctoral researcher in the group, has also obtained funding to start a project on genomically minimal cells, bacteria where all non-essential genes have been removed.

We have also been working on epidemiological models (with Susanna Manrubia) and control of organ size during embryonic development (with Fernando Casares, CABD).

Finally, we have been actively analysing COVID-19 data on Twitter through Saúl Ares account @omeuxito.

1



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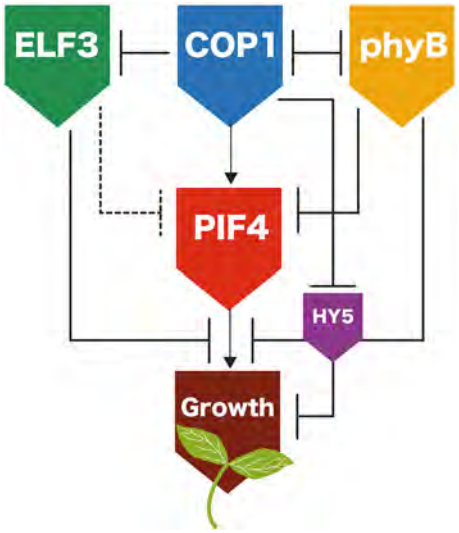

1 *Simulation of an Anabaena filament under three different conditions. The importance of boundary conditions is apparent in the terminal cells of patA mutant filaments.*

2 *Regulatory network controlling the effect of light and temperature on hypocotyl elongation in* Arabidopsis thaliana.

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Casanova-Ferrer P, Muñoz-García J, Ares S. Mathematical models of nitrogen-fixing cell patterns in filamentous cyanobacteria. Front Cell Dev Biol 2022, 10, 959468.

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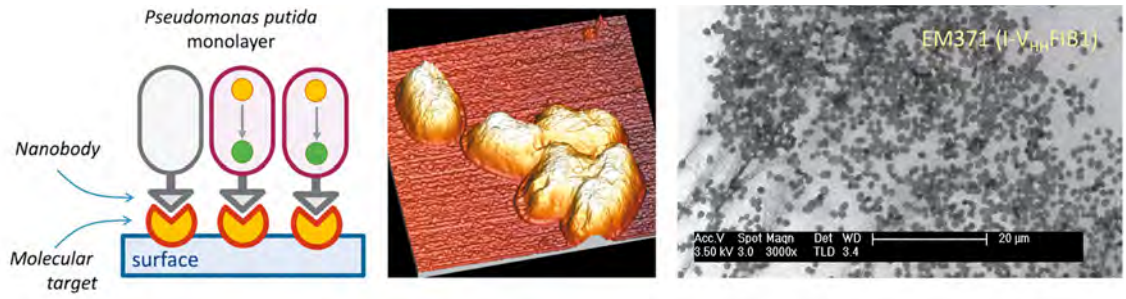
Environmental synthetic biology

The longstanding mission our team is the production of biological agents for biosensing, remediation and (wherever possible) valorisation of chemical waste that is otherwise dumped into the Environment by urban and industrial activities. The workhorse to this end is the soil bacterium *Pseudomonas putida*, which combines the ease of genetic programming that is typical of *Escherichia coli* with the safety, robustness and metabolic capabilities required in whole-cell catalysts for applications in harsh biotechnological settings. Specific activities include: [i] Development of *P. putida* as a reliable chassis for implantation of genetic and metabolic circuits. This involves a profound editing of the extant genome of this microorganism for enhancing desirable properties and eliminating drawbacks. Also, the exploitation of surface-display systems for designing complex catalytic properties altogether separated from the cell metabolism and even the design of artificial communities by means of ectopic adhesins. [ii] Genetic tools for deep refactoring of metabolic properties of *P. putida*. The list of new assets that we are developing includes a large collection of standardized plasmid and transposon vectors. [iii] The TOL system borne by plasmid pWW0 as a reference for metabolic circuit implantation. The two operons for toluene and m-xylene biodegradation encoded in pWW0 offer a natural case of expansion of the metabolic repertoire of environmental bacteria through acquisition of new genes. [iv] Deep metabolic engineering of *P. putida*. Our long-term ambition is engineering propagation of the thereby designed bioremediation agents at a very large scale much beyond Laboratory, bioreactor or microcosm setups, for which we are placing a considerable effort in domestication of horizontal gene transfer.



Strategy for generating monolayers of Pseudomonas putida *cells specifically stuck to a solid surface by means of ectopic display of single-chain camel antibodies (nanobodies).*

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Evolutionary systems

The main research topic of the group is the understanding, modelling and analysis of evolutionary mechanisms in biological and social systems. For almost two decades, we have investigated the adaptive dynamics of viruses and RNA populations and addressed broader problems, such as the relationship between genotype and phenotype (figure 1). Recently, we have explored the topological structure that genotype-to-phenotype maps endow in sequence spaces, and its effects in the dynamics of heterogeneous molecular populations. We have uncovered some universal features of sequence spaces topology which are independent of the definition of phenotype and, therefore, have generic consequences for evolution and adaptation. Our results highlight the role of entropic effects in microscopic evolution: abundant, sufficiently functional phenotypes, are much more common in nature than highly adapted, but rare ones. A full understanding of microscopic evolution is important to update current evolutionary theories and to derive useful effective models. In this sense, we question the role played by classical metaphors of evolution, and suggest that smooth fitness landscapes must be substituted by network-based representations. General evolutionary and adaptive processes affect multiple disciplines beyond biology. Game theory, understood as the search for strategies that optimise fitness, can be applied to economic and other processes involving agents able to take decisions. In rigged economies, where market rules allow agents to artificially modify stock market prices, we have shown that economies increase in complexity: while growing economic complexity spontaneously defuses cartels, it also leads to large-fluctuations regimes that threaten the system's stability. In the context of epidemic propagation, we have shown that, even in the absence of non-pharmaceutical measures, epidemic waves and a convergence towards the critical propagation rate (figure 2) can originate from a self-adapting population behaviour, where individuals vary their degree of exposure according to their subjective perception of the external threat.

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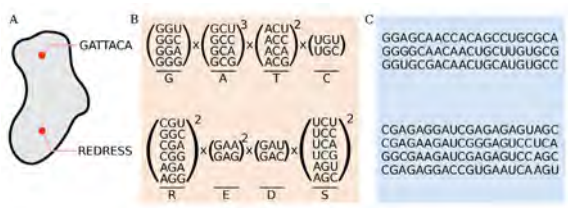

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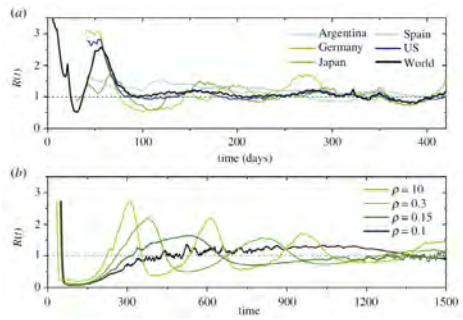

1 *Illustration of phenotypic redundancy in a neutral nucleotide-to-amino-acid-sequence model. (A) The space of protein sequences of the same length is vast and contains a variety of functional sequences. (B) The number of codons representing each amino acid varies: codons coding for glycine (G), alanine (A), threonine (T), cysteine (C), arginine (R), glutamic acid (E), aspartic acid (D), and serine (S) are shown here explicitly as examples. (C) Possible nucleotide sequences coding for GATTACA (above) and REDRESS (below). From Villanueva et al., Biophysica 2022.*

2 *Effective reproduction number R_0 as a function of time. (a) Empirical estimation of COVID-19 R0 for various countries and the World since 23 January 2020. (b) Evolution of R_0 value for a model that incorporates the risk-aversion response of individuals to the pandemic state. The model generates epidemic waves and a value of R_0 around 1, in agreement with natural progression (from Manrubia and Zanette, RSOS 2022).*

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Systems Biotechnology

Our foundational aim is the system-level understanding of microbial metabolism as a framework for developing a broad range of novel and non-intuitive biotechnological processes. Taking advantage of metabolic modelling, systems and synthetic biology we are addressing, at different levels, the understanding and full taming of bacterial systems emergence.

Increasing the completeness and scope of metabolic reconstructions

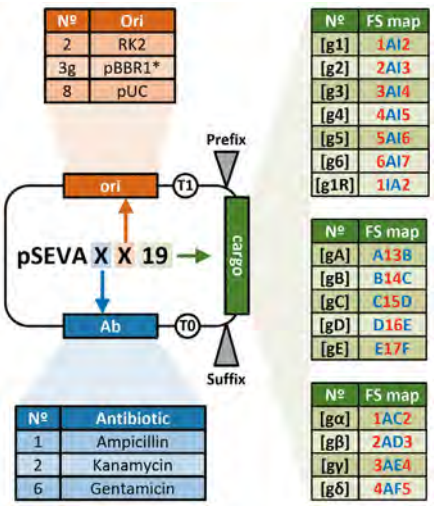
We are involved in the high-quality metabolic modeling of a large set of metabolically diverse bacteria including *P. putida, S. elongatus, A. platensis, Azoarcus* CIB, *S. granuli, P. pseudoalcaligenes, B. bacteriovorus, H. influenzae and Bifidubacterium sps*. This effort is enabling the system-level analysis of new metabolic processes while providing new computational test-beds for biotechnological applications. We are particularly interested in the inclusion of new metabolic modules such as the generation of reactive oxygen species, underground metabolisms and metabolic heterogeneity.

System-level analysis of Metabolic Robustness in bacteria

The robustness of a system is the property that allows it to maintain its functions despite perturbations. Through the metabolic modeling analysis of P. putida, we have identified metabolic cycles providing robustness. The synthetic biology assisted validation of such cycles is allowing the rational engineering of superior microbial biocatalyst under diverse biotechnological scenarios.

System-level analysis and designing of microbial communities

The division of labor allows an expanded complexity and functionality in bacteria. We are interested in: i) understanding how these expanded capabilities emerge within a bacterial populations and communities and ii) how we can engineer this supracellular-level functionality towards biotechnological endeavors. To address these two fundamental questions, we have developed systems and synthetic biology tools for modeling and engineering synthetic microbial populations and consortia. We are applying this technology in the revalorisation of complex polymers such as lignin and plastic waste as well as in the cost effective production of plant-based secondary metabolites such as flavonoids.



Structure and nomenclature of Golden Standard pSEVA vector collection for modular cloning developed at Systems Biology Group. Golden Standard cargo is denoted with the number 19.

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Manoli MT, Nogales J, and Prieto A. Synthetic control of metabolic states in *Pseudomonas putida* by tuning polyhydroxyalkanoate cycle. Mbio 2022, 13 (1), e01794-21.

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Computational systems biology

In the last two years, we continued working in the application of network-based approaches to the study of human pathologies. Within this line, in collaboration with the group of Prof. Juan. A.G. Ranea (U. Málaga), we worked on the systematic identification of genetic systems associated with phenotypes in patients with rare diseases. Along the same line, we also developed a novel methodology for the comprehensive detection of relationships between biomedical concepts in the scientific literature, using a co-mention based approach. Along our research lines dealing with protein structure and function, we developed a methodology for finding protein sites related to interaction specificity, as well as a new approach for the concomitant detection of residues and physicochemical properties related to functional specificity. Within our Systems Chemoinformatics research line, we finished the development of a profile-based approach for assigning chemical compounds to functional classes.

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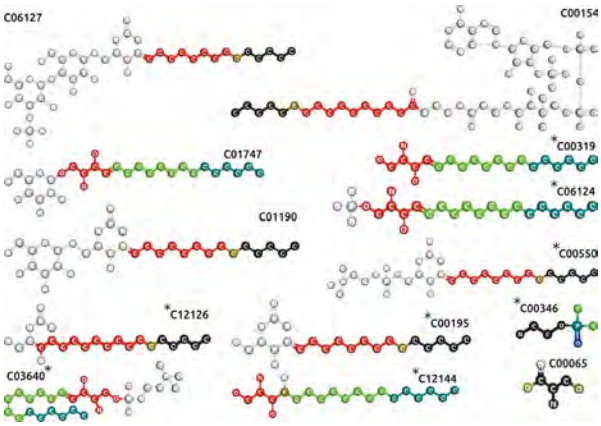

1 *Chemical fragments determining the pertenence of chemical compounds to the "sphingolipid metabolism" KEGG pathway KEGG (map00600), highlighted in the structures of the compounds of that pathway.*

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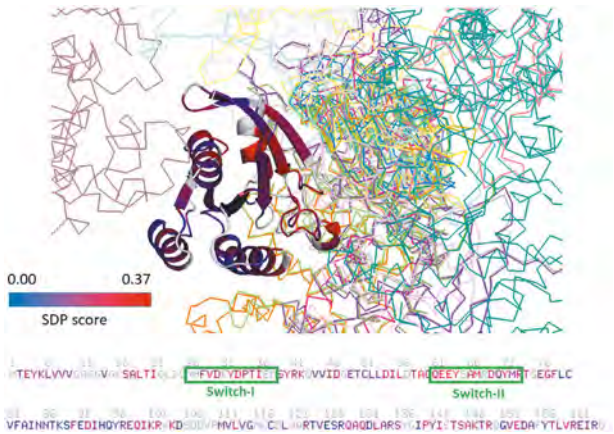

2 *Prediction of regions determining interaction specificity for RasH, mapped on the interaction structural information available for that protein. RasH is shown in ribbon representation, and its 26 crystallised interactors in thin backbone. The method's score for the RasH residues is shown in a color scale, with red representing the highest scores.*

SELECTED PUBLICATIONS

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Pazos F, Chagoyen M, Seoane P, Ranea J. CoMent: relationships between biomedical concepts inferred from the scientific literature. J Mol Biol 2022, 434(11), 167568.

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Logic of genomic systems

How does a biological system deteriorate? Genetic mutations could be a dominant factor. Are there ways to buffer the function of the system against these types of disturbances? By examining genome-wide expression patterns in response to gene deletions, we found that responses are stereotyped, and in some cases do not buffer but rather potentiate functional disruption resulting from the deletion itself.

This result made us wonder to what extent we could anticipate the functional impact of a mutation. We studied this problem using *E. coli* RNA polymerase (RNAP) as a model. RNAP mutations can affect multiple phenotypes that are apparently unrelated. We examined the direct effects associated with RNAP function but also the system-level response that causes additional "indirect" effects. Our work proposes that an important driver of the functional costs produced by mutations is the indirect effect of altering the so-called global transcriptional regulatory program; a program that connects the physiological state of the cell with its gene expression.

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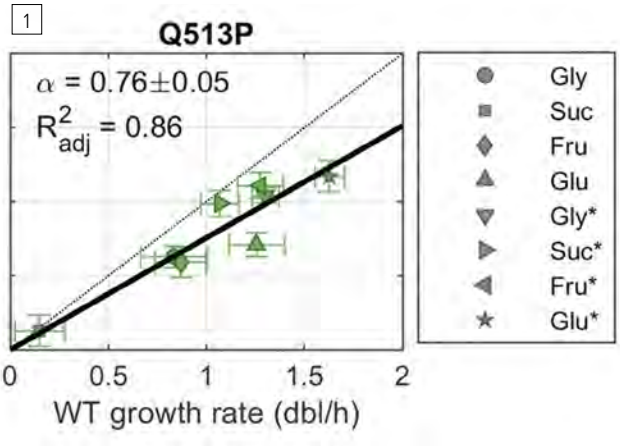
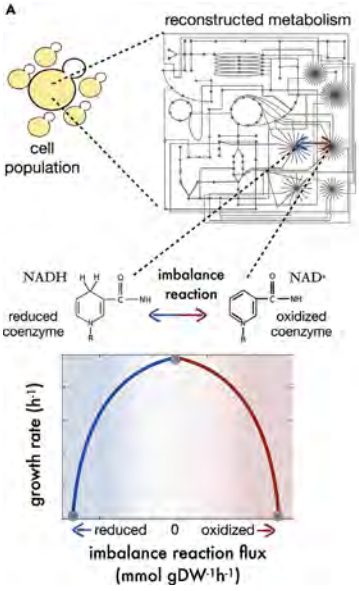


1 *The growth rate of a rpoB Q513P mutant strain and its relative WT in eight different growth media shows a global fitness cost.*

A second way of analysing how a biological system deteriorates is by focusing on the appearance of metabolic imbalances. To study this, we focused on the NADH/NAD+ redox couple in yeast. Using large-scale metabolic models, we showed that reductive imbalances generate metabolic syndromes comparable to those seen in cancer cells and identify the underlying mechanisms of pathology, lifespan-protecting molecules, or caloric restriction mimetics. Tolerance to redox imbalances thus becomes a robust framework for recognising deteriorating system properties while providing a consistent biological rationale for evaluating protective interventions.

Work during this period has led us to be interested in understanding the limitations in prediction and causal inference in biological systems. We did a sabbatical in the CSIC Institute of Mathematics (ICMAT) that will have a sure impact on our research at the Logic of Genomic Systems Laboratory in the coming years.

2



2 *We can control the interconversion of the two conjugated forms of a NADH/NAD+ pair in a yeast in silico metabolic model and then apply flow balance analysis to calculate growth rate. The predicted values of growth are plotted against the values of the rate of this imbalance as an indicator of the tolerance of metabolism when faced with the chosen perturbation*

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Microbiome analysis

Microbial communities (microbiomes) are key players in many scenarios, from how the biosphere works to industrial and biotechnological processes, as well as human health and wellness. We study microbiomes of diverse environments trying to learn the rules that govern the assemblage of these microbial communities. This knowledge will help to understand how they function, and to predict the effects of disturbances. Eventually, this will lead to rational design and manipulation of microbiomes.

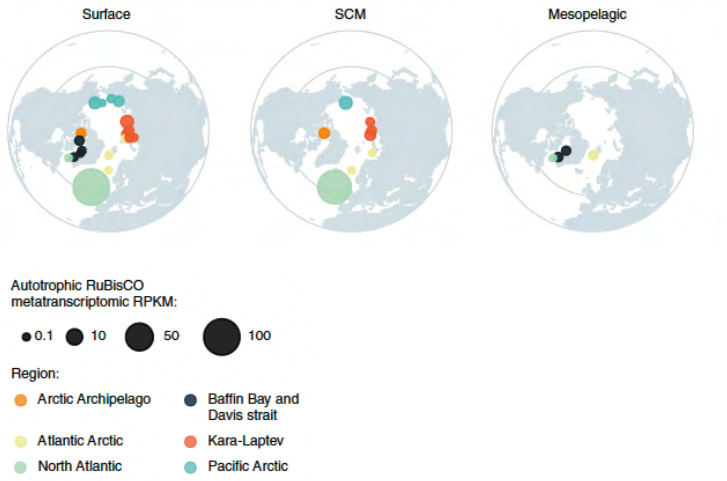
We focus mostly on marine microbial communities, but we are actively working in many other microbiomes from different environments. We study extreme environments because their microbiotas show fascinating adaptations to the harsh conditions. We work with human-associated microbiomes, such as the gut and the vagina, because of their potential to improve our health. We are also interested in other habitats, such as wastewaters and soils.

We use mostly bioinformatics tools to study the composition and functionality of microbiomes. Metagenomics is the basis of our work, since it provides the basic material: DNA sequences from environmental samples. The analysis of these sequences informs about the presence of diverse organisms and the content of their genomes, and the latter can be linked to functionality. We also carry out experimental work addressing interactions between members of microbiomes.

1

1 *Testing the* in situ *protocol for sequencing and annotation of metagenomes in La Palma lavas.*

2



2 *Gene expression in the TARA Arctic cruise. Transcript abundance of RuBisCO forms I and II, involved in the Calvin cycle pathway (K01601), color-coded by Arctic region. The size of the dots is proportional to the accumulated metatranscriptomic RPKMs.*

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Peña-Ocaña BA, Ovando-Ovando CI, Puente-Sánchez F, Tamames J, Servín-Garcidueñas LE, *et al*. Metagenomic and metabolic analyses of poly-extreme microbiome from an active crater volcano lake. Environ Res 2022, 203, 111862.

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